CLAIMS

1. A method for generating calibration data for absolute quantitation of RNA by RT- PCR, the method comprising: (a) providing a synthetic oligonucleotide comprising an amplicon and a promoter sequence located 3' relative to the amplicon; (b) synthesizing complementary RNA (cRNA) by *in vitro* transcription of the oligonucleotide; (c) quantitatively assaying the cRNA by an independent method; and (d) generating calibration data using a known quantity of the cRNA.

2. The method of claim 1, wherein the promoter sequence is a bacteriophage promoter sequence.

3. The method of claim 2, wherein the bacteriophage promoter sequence is a T7 promoter sequence.

4. The method of claim 3, wherein the T7 promoter sequence consists essentially of 5'CCTATAGTGAGTCGTATTA 3' (SEQ ID NO:1).

5. The method of claim 1, further comprising a 5' flanking sequence consisting of 2 to 20 nucleotides adjacent to the amplicon.

6. The method of claim 5, wherein the 5' flanking sequence consists of 8 to 12 nucleotides.

7. The method of claim 5, wherein the 5' flanking sequence comprises a poly T tail.

8. The method of claim 1, wherein the synthetic oligonucleotide further comprises a 3' flanking sequence consisting of 2 to 20 nucleotides between the amplicon and the promoter sequence.

9. The method of claim 8, wherein the 3' flanking sequence consists of 8 to 12 nucleotides.

10. The method of claim 1, wherein the length of the amplicon is 30 to 70 nucleotides.

11. The method of claim 10, wherein the length of the amplicon is 40 to 60 nucleotides.

12. The method of claim 1, wherein the length of the synthetic oligonucleotide is 60 to 140 nucleotides.

13. The method of claim 12, wherein the length of the synthetic oligonucleotide is 70 to 130 nucleotides.

14. The method of claim 13, wherein the length of the synthetic oligonucleotide is 80 to 120 nucleotides.

15. The method of claim 14, wherein the length of the synthetic oligonucleotide is 90 to 110 nucleotides.

16. A method for determining the abundance of nucleic acid molecules comprising an amplicon in a test sample, the method comprising:
(a) providing a synthetic oligonucleotide comprising an amplicon and a promoter sequence located 3' relative to the amplicon;
(b) synthesizing cRNA by *in vitro* transcription of the oligonucleotide;
(c) producing a dilution series using the cRNA;
(d) synthesizing single stranded cDNA by reverse transcription of the cRNA;
(e) generating RT-PCR calibration data;
(g) obtaining RT-PCR test sample data from the test sample; and
(h) comparing the PCR test sample data to the PCR calibration data.

17. The method of claim 16, further comprising quantitating the cRNA.

18. The method of claim 17, further comprising mixing the cRNA with heterologous RNA before synthesizing the single stranded cDNA.

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